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YEASTS AND THEIR RELATION TO MALIGNANT TUMORS.

ALLEN ROSS DEFENDORF, A. B., M. D., WORCESTER, MASS.

Since the possibility of a parasitic origin of cancer, as well as other tumors, was suggested by Paget,¹ who likened the growths produced upon trees by the stings of insects to the tumors of men, almost every pathologist of any renown has investigated the subject.

A critical investigation of the voluminous literature on this subject impresses one with the fact that something is at fault, for when almost all are united in asserting the presence of "Cancer Bodies," yet few have dared to tell us just what they are. The fault lies in the fact that, with a very few exceptions, research has been conducted only on a historical basis.

Six years of investigation have shown that from the morphological standpoint, there is no hope of establishing the specific cause of these neoplasms, for, since the promulgation of Koch's laws, scientists in mycology have been loath to accept as the cause of disease, organisms which do not conform to its requirements.

The recent discovery of the fact that the class of fungi called yeasts are pathogenic has opened up a new field.

Since this discovery by Sanfelice, in 1893, the subject has aroused much interest, especially among the Italian and German pathologists, so that now the yeasts which meet the requirements of Koch's laws are to receive a large share of the attention which was so lavishly bestowed upon the supposed protozoan parasite.

Yeast fungi, also called *Blastomycetes*, are the chief representatives of the genus *Saccharomyces*. It is to be regretted that their exact botanical position has not been determined.

Formerly they were considered as a stage in the development of filamentous fungi, but now authorities agree that they form a distinct class.

Morphologically they are round or oval cells, arranged singly, or in chains, increasing by budding or by spores, and occasionally forming mycelia. Each cell consists of a mass of granular protoplasm, surrounded by a double contoured membrane. The younger forms are, for the most part, homogeneous, while the older forms contain one or more refractive granules.

The cells as they are seen in animal tissues are somewhat different, in that they usually possess refractive granules, and a membrane varying in thickness. In some cases they are surrounded by another double contoured hyaline membrane. The protoplasm is usually hyaline. The mother cell rarely gives rise to a mycelium, which is considered an abortive form.

Much discussion has arisen in regard to these refractive granules, whether or not they are or represent nuclei. Raum says that he could never establish a nucleus. Sanfelice says that the granules cannot be compared to such a nucleus as we find in a fully organised cell. Though they stain more deeply than the protoplasm, they cannot on account of their lack of consistency be called nuclei, and besides, they have never been seen to take part in the process of budding (excepting Sirleo and Maffucci). They are persistently present in all kinds of *Blastomycetes*, but they present no constant attributes as to form, arrangement, number or changes. They were observed by Schwarm and de Legues, also by Rees and Hausen, and were called endospores, a view which Raum, Möller and Brefeld justly opposed.

Yeasts stain equally well with any of the aniline dyes, also with carbofuchsin, and hematoxylin (Büsse). The methods of staining are as follows :

The tissue is stained in toto with lithium-carmines for twenty-four hours, twenty-four hours in alcohol acidified with $\frac{1}{2}$ per cent of hydrochloric acid, treat with absolute alcohol as long as stain is removed, clear with xylol, imbed

in paraffine, free sections of paraffine with xylol, treat with absolute alcohol, let stand from five to fifteen minutes in a flask filled with Ehrlich's solution, wash with water, treat with a few drops of 5 per cent. oxalic acid, which acts as a mordant, wash off the acid with water, treat with absolute alcohol as long as the color is removed, clear in xylol, mount in balsam.

By this method, which was selected by Sanfelice because of the beautiful colors, the tissue ground work is stained red and the parasite violet.

In Ehrlich's fluid, methylene-blue or malachite-green can be substituted for gentian-violet, when the parasites are colored respectively blue and green. If the tissue is stained in sections, the following is a good method.

The section freed of paraffine is stained ten to fifteen minutes, or one to three hours in the following solution :

Saturated watery solution of Safranine	1 p.
Saturated alcoholic solution of Malachite-green	$\frac{1}{2}$ p.
Distilled water	$\frac{1}{4}$ p.

Wash in distilled water, treat five minutes with 5 per cent. oxalic acid, wash, treat with absolute alcohol, clear in xylol and mount in balsam.

By this method the parasite is stained green or bluish-green according to the length of time the tissue is allowed to stand, the protoplasm flesh-red and the nucleus safrin-red.

In place of the staining fluid, mentioned in the above method, the following solution may be substituted :

Saturated watery solution of Safranine	5-6 p.
Saturated alcoholic solution of Gentian-violet	1 p.
Distilled water	$\frac{1}{4}$ p.

Leave the specimen in the staining fluid three or four minutes, which stains the parasite violet and the tumor the high reds of safranine.

Good results can be obtained from a mixture of borax carmine and indigo carmine. After staining, the sections are treated with a saturated solution of oxalic acid. The parasites stain blue and the tumor red.

Gram's method may be used, but the solution of iodine must be very weak, so as not to decolorise too strongly.

Yeasts are widely distributed throughout nature. Brefeld² found them in air, dust, plants, leaves, fruits and manure. The most difficult part in the study of these fungi has been to get them to grow on nutrient media and to isolate them, so as to procure pure cultures. Their behavior in culture growth resembles very much that of bacteria, which exhibit a few characteristics in common, but present individually their peculiar reactions in different media. They prefer acid media, in which they grow very slowly. They do not liquefy gelatine. Potato is a favorite medium, upon which their growth is usually luxuriant. A common characteristic which most of them possess is the ability to produce alcohol in sugar cultures. An idea of the manner of their growth on the different media can be obtained in the study of some of the pathogenic varieties. They do not produce toxins, but those which produce pathological effects do so by means of general infection.

Since the discovery of the yeasts, in the early part of the century, they have been identified with the process of fermentation, in which they are the active agent. It was not known until the studies of Newmayer³ and Raum⁴ that they possessed pathogenic qualities.

Raum, who inoculated ten rabbits seventeen times with the *Cereveus* and the *Ellipsoideus*, found that death resulted when large quantities were inoculated. Quantities of the parasites were found post-mortem in the lungs, where they had produced thrombosis. In one instance, a tumor was produced in the ear of a rabbit injected intravenously, which proved a caseous mass containing fungi.

Sanfelice,⁵ although he had done much work on the morphology of *Blastomycetes*, as he prefers to call them, did not study their pathological effects until the latter part of 1894, when he found that a certain variety which he had isolated was pathogenic in pure cultures for guinea-pigs, sheep, rabbits, rats, slugs and pigeons.

Because of the similarity of the colonies of this *Blastomy-cete* on solid media to those described by Cuboni and Pizzigoni as characteristic for the *Saccharomyces ellipsoideus*, Sanfelice placed them in the genus of *Saccharomyces* and on account of the pathological changes he called them *Saccharomyces neoformans*.

The culture growths were as follows: On gelatine plates the surface colonies, about the size of a pin-head and cup-shaped, were white in color, while the colonies below the surface were smaller, spherical, with a yellowish-white color and a well-defined margin. The medium was not liquefied, and whether neutral or acidified with 1 per cent. tartaric acid, offered an equally good soil. Under a microscope of high power, the cells were easily distinguished, packed very closely together. Colonies on agar plates presented the same appearances as above. In gelatine stab cultures the growth was luxuriant, both on the surface and along the stab. On the surface of slant agar, neutral or slightly acidified, they produced a finely granular, dry, non-glistening membrane, which very slowly covered the whole surface. On the potato it had the appearance of a white, lusterless membrane, with warty prominences, but a smooth border. The growth was luxuriant in milk, but produced no change in the medium. In fluid media, to which different kinds of sugars had been added—glucose, maltose, dextrose, lactose and saccharose—there was a rich growth, producing no turbidity, but oftentimes there was found on the surface a more or less thick white membrane. Dried preparations of media, stained with the watery or alcoholic solutions of aniline dyes, showed the greater number of cells to be stained intensely, throughout their entire structure. A few were stained more intensely at the periphery, while one large or several small deeply stained granules lay in the center. Examined in the hanging drop, these cultures by careful observation showed the process of budding.

The observations of Sanfelice upon the pathogenic qualities of this variety of yeast were made from inoculations of

guinea-pigs with pure cultures. The fungi were scraped from the surface of an agar or potato culture, mixed with a small quantity of sterilised water and immediately inoculated. Cultures from each of the solid media were equally virulent. Age had no effect upon the virulence, for cultures five months old were no more virulent than fresh cultures. In all, forty guinea-pigs were inoculated subcutaneously, into the testicles, into the liver and into the peritoneal cavity, with fatal results. Those which ingested the pure culture showed no apparent effect. With the exception of the last, all died in about thirty days, only two living longer; one forty-three days, and another seventy-five days. Of those inoculated subcutaneously, there appeared in the adjacent connective tissue a tumor, which was as large as a hazel nut. Within a short time after the inoculation, the lymphatic glands in the inguinal and axillary regions began to enlarge. At the autopsy the tumor seemed to be composed of a firm white tissue and was adherent to the skin. The lymphatic glands of the axillary and inguinal regions were greatly enlarged and exhibited a tissue similar to that of the tumor. Upon opening the abdomen the spleen was found to be enlarged and covered with white spots. The liver and lungs were enlarged and with the kidney, showed similar white spots on their surfaces.

Guinea-pigs inoculated in the testicles showed at the autopsy swellings of that organ, together with changes in the abdominal cavity, much the same as those of the animals inoculated subcutaneously. In the guinea-pigs inoculated in the peritoneal cavity, the organs of the abdomen and thorax were more profoundly involved than in those inoculated subcutaneously or in defined organs. In the cavity there was a severe peritonitis, which might have been called neoplastic, and the surface was covered with a milk-white fluid. Tumors the size of a hemp seed, white in color, were found on the parietal peritoneum and the omentum. The lymph glands were enlarged.

The tissues of the guinea-pigs, when fixed, hardened,

stained and mounted, showed interesting changes. The tumors at the site of the subcutaneous inoculation presented a nest of young connective tissue, in the meshes of which a varying number of cellular elements, which were very similar to lymph corpuscles, and whose nuclei laid mostly at the periphery of the cell body. Among these elements there were so many parasites that the tumor seemed to be due rather to the presence of the parasites than to the tissue elements.

The fungi were mostly free, and well stained, though a few were without a trace of stain. Their size varied greatly. Among the very young forms no membrane was seen; in forms of medium size there was a hyaline membrane, and in the oldest forms there was a deeply stained membrane. The last forms correspond in the fresh preparations to the forms with strongly refractive membrane.

The granules were usually deeply stained, though in some cells which had undergone degeneration nothing within the membrane had taken on stain. The protoplasm of some cells showed very clearly the two kinds of protoplasm stained to a different degree. Among the very large forms were seen some with a very clear hyaline membrane outside, but no trace of an inner membrane.

The tumors of the guinea-pigs inoculated in the liver seemed due rather to the presence of large numbers of the parasites than to any great change in tissue elements. Aggregations of yeasts corresponded to the white spots and granules scattered on the surface of the organ. These groups were found not only under the capsule but also in the parenchyma.

The tumor of the great omentum in the guinea-pigs inoculated in the peritoneal cavity presented the same structure as those noted above—namely, numerous fungi lying between the meshes of a loose connective tissue, which was in some places thickened, but otherwise normal. Many parasites were found also in the pancreas and salivary glands.

In the kidneys they were more numerous in guinea-pigs

inoculated into the peritoneal cavity than into the subcutaneous tissue or into special organs. The situation by preference seemed to be in the cortex and inside of the increased connective tissue, corresponding to the white spots seen by the naked eye. Groups of the parasites had destroyed parts of the convoluted tubules. They were seen in the loops of the glomeruli, and, as a consequence of the rupture of the glomeruli, had escaped into Bowman's capsule. By this it was proved that in the kidney, at least, they spread through the blood.

The number of parasites was much larger in the lungs of the guinea-pigs inoculated in the peritoneal cavity than subcutaneously. They were found in larger numbers in the lungs than in any other organ. They laid in the spaces between the alveoli, and caused a considerable increase of tissue.

This variety of yeasts was of less importance on account of the pathological conditions which it produced than because of the morphological similarity which its forms presented to the structures, which have been described by the many authors on malignant tumors of men as belonging to the Protozoa.

Sanfelice has since discovered several varieties of yeasts, which he has not yet described fully, but which he claims belong to the group of *Neoformans*. Whether or not they are pathological he has not determined. He has, however, succeeded in two cases, one of an epithelioma of the lip and the other in an epithelioma of the mammary glands, in securing pure cultures of yeast fungi, which he has proved by inoculations to be pathogenic to animals.

Sanfelice⁶ remarks upon the neoplastic growth in a slut inoculated in the mammary glands with a pure culture of another yeast, that a tumor was produced at the site of inoculation, and also that there were metastatic tumors in the intestines and in the spleen. In the connective tissue which surrounded the gland, groups of cells were noticed, some of which possessed a large nucleus pressed to the peri-

phery, and other smaller nuclei similarly disposed. The cells were packed together in rows which crossed each other, giving the appearance of a fibrous carcinoma. From these groups radiated outward rows of cells which seemed to burrow between the connective tissue fibers. There was no evidence of inflammation. The parasites could not be found in the center of these groups, even by the most careful searching. Cells at the periphery, however, contained them, where they appeared to be in the early stages of growth, mostly without membranes. The histological structure of the new growths in the other organs was of a similar character to that in the breast. The yeasts were situated in the cytoplasm of the cells, were round, and possessed the characteristic double contoured membrane. With this variety an inoculation was made between the leaves of the comb of a rooster, and caused a neoplasm, which approached in character a sarcoma. The sections of the tumor showed that the larger number of the yeasts was in the central part of the tumor. Many of the parasites were degenerated, and so stained lightly. The tissue of which the tumor was composed consisted of young connective tissue elements, among whose bundles lay many cells with intensely stained nuclei and sharply defined cell bodies. In some places these cells produced thick bands, among which could be perceived isolated yeast cells.

In May, 1895,⁷ Sanfelice isolated a variety of yeasts from an ox which had died of primary cancer of the liver and secondary invasion of that process into the whole lymphatic system. Pieces of the enlarged glands were applied to the different fluid and solid media and a culture established. By teasing pieces of the glands in a fluid consisting of equal parts of glycerine and water, yeasts were found in large numbers, of different sizes, mostly round and possessing a refractive membrane. Most of them contained refractive granules. Near these parasites were found many others of an appearance which the *Blastomycetes* ordinarily give inside of the cells. They were homogeneous and of a glassy refrac-

tibility, or of that sort of refractibility which calcareous bodies present in pathological tissues. Besides these forms there were irregularly formed masses with a sharp outline and a calcareous refractibility, which he at first considered to be calcareous degeneration products.

Plate cultures of gelatine showed colonies of yeasts which on the surface were round, white and of the size of a pin-head, but beneath were smaller, spherical, sharply defined and of a yellowish color. Stab cultures in gelatine showed colonies both on the surface and along the stab. On slant media the colonies were white, elevated and of a dried appearance. Examined microscopically, they appeared as round cells, of different sizes, according to the age of the culture, and with glistening granules ; but the glassy-looking bodies, described above, could not be found,

From the culture growths and the microscopical appearance, the yeast seemed in no way different from the pathogenic variety, named *Saccharomycetes neoformans*, and described above.

Guinea-pigs inoculated subcutaneously with an emulsion of pieces of the glands and sterilised bouillon died in two months and showed the following pathological conditions :

The axillary and inguinal lymph glands were enlarged ; the great omentum showed a few small nodules the size of a hemp seed ; a few were seen also in the spleen ; the lymph glands of the abdomen were also enlarged ; small nodules were seen in the lungs ; the liver, intestines and brain exhibited no changes. When inoculated intraperitoneally, conditions identical to those just described were found.

Small pieces of the lymphglands of the dead guinea-pigs, teased and examined fresh under the microscope, showed parasites both free and enclosed in the cell elements of the gland. They disclosed the characteristic membrane, while some of them had a more or less hyaline halo surrounding the membrane. The protoplasm was homogeneous with the usual number of refractive granules. Besides these normal forms were seen others, which refracted the light like

glass. These were degenerate forms of the fungi. In the kidney they were present in such numbers that upon section it seemed as if the organ was full of calcareous deposits. It was noticed with those guinea-pigs which died a greater number of days than usual after the inoculation that the reaction on the part of the tissue was greater, from which fact it was concluded that after some time, if the animal survives, the parasites tend to degenerate and disappear.

Sanfelice gave this variety of yeast fungi the name of *Saccharomycetes litogenes*, from the appearance of calcareous degeneration which it gave. The *Saccharomycetes litogenes* were pathogenic also for white rats, rabbits, sheep and cattle.

Shortly after the first contribution of Sanfelice appeared, Maffuci and Sirleo⁸ described a pathogenic yeast fungus which they obtained from a guinea-pig that had died of marasmus. They were successful in establishing pure cultures, which upon inoculation into guinea-pigs gave fatal results.

The parasites were found in the lung, kidney and lymph glands. They observed that the structure of the cellular tissue was the same in all of the above organs, and similar to that which was described above, in Sanfelice's investigation. They noticed, however, a greater activity in the formation of new structure in the lungs than in the other organs. This dissimilarity may be explained by a longer time, which the parasites had acted before the death of the animal, which fact was not mentioned.

Otto Büsse⁹ discovered a pathogenic fungus in the study of a malignant tumor of the tibia of a woman, which during life was diagnosed as a sarcoma, but by post-mortem examination appeared as a form of chronic pyemia, with a number of giant cells. They appeared in the pus among the giant cells as small, clear, refractive, round or oval bodies, of a size varying between that of a small nucleus and a liver cell. They were also found as cell enclosures within the giant cells. At first, they were looked upon by Professor Grawitz as belonging to the group of Microsporidia, being

Cornalia's bodies or *Coccidia*. Later, as the result of positive culture, they were identified by Professor Löffler as a kind of pathogenic yeast fungus. The possibility that he might be dealing with a variety of *Coccidia*, as the descriptions and drawings of Darier and Wickham suggested to him, led him to try a culture in sugar bouillon, which produced alcohol. This left no doubt that the parasites were yeast fungi.

He injected into the marrow of the tibia of a rabbit a small piece of the tissue taken from the tumor. The leg became gangrenous in three days and was amputated, when it was found that the whole length of the tibia was beset with yeast cells, which proved that the yeast cells had the ability to multiply in human tissues. Some of the pus from the tumor was injected into the peritoneal cavity of a rabbit, with fatal results in three days. The mesenteric glands were much enlarged and in them were found the parasites in large numbers.

The fact that Büsse published his results a month after his first inoculation, before some of his test animals had begun to show the effects of the inoculation, or before their tissues had been examined histologically, prevented him from drawing any other conclusion than that the yeasts were capable of multiplying in animal tissues.

Ajevoli¹⁰ and Pianese¹¹ have done work which could not be obtained.

Corselli and Frisco¹² succeeded in isolating a pathogenic yeast from a case of sarcoma of the mesenteric glands with a milky exudate. At the autopsy a neoplastic mass was found, consisting of enlarged mesenteric glands with many small tumors, of the size of a lentil, situated on the diaphragm and small intestines, and a quantity of milky fluid in the abdomen and thorax, similar to that seen during life. Microscopical examination of the fluid, made during life, showed cellular forms of different sizes, some small, others the size of a liver cell, mostly round, which were isolated or in groups, very often of four. Most of them showed one or more granules. They stained by the usual methods.

Inoculations of the pathological material were made upon the ordinary media, but neutral or alkaline focus was the only one upon which a culture grew. From this medium colonies of the *Blastomyces* were isolated and transferred to gelatine, simple agar, glycerine agar, and sugar agar, and also bouillon, where the culture flourished. A microscopic examination of the pure cultures showed the fungus to be of a larger size than those in the pathological material, though in respect to the refractive granules no difference was noticed. The cells were surrounded with a thin membrane. The protoplasm was usually homogeneous, with a granular appearance at some places. In the hanging drop were seen small granules, which increased in size and approached the appearance of round bodies, and which later became still more prominent on account of their different refractive contour and their different disposition toward stains. Finally, they burst the membrane and became free, assuming the first stage of the parasite. The more usual means of propagation was that of budding. Another unusual form was that of forming a rod-like appendix, which showed the tendency for producing a mycelium.

The results of the inoculation of guinea-pigs, rabbits and dogs, with both the pathological material and the pure cultures, were uniformly fatal. The guinea-pigs inoculated with five cubic centimeters of the milky fluid, or two cubic centimeters of a ten-day bouillon culture, died in about thirty days. The lymphatic glands of the mesentery were greatly enlarged. At other places in the mesentery appeared numberless groups of ball-like nodules. Similarly many little nodules were found along the line of the lymph channels, in the axillary and inguinal regions. A microscopical examination of these nodules disclosed a structure similar to that found in the woman, except that mycelial forms of the fungi were very rare. Rabbits inoculated with five cubic centimeters of the fluid, or five cubic centimeters of the bouillon, died in from thirty to thirty-five days. The same appearance, macroscopically and microscopically, were noted in the guinea-pigs.

Of the dogs which were inoculated intravenously and intraperitoneally, one died in five days without any apparent changes. The parasite was regained only from the blood of the heart. One of the dogs inoculated intraperitoneally, showed a tumor of the mesentery, the size of a small hen's egg, and lymph nodules as large as white beans along the spine and in the peribronchial glands.

The facts that negative results were obtained in the cultures of the parasites in the juices from fruits, that there was a slight tendency to grow on acid media, and a failure to produce alcohol in saccharin fluids, influenced the investigators to conclude that during their life as parasites in the tumors of animals, they naturally suffered a change in their mode of life, and acquired a character qualified for their parasitic existence in animals.

From these observations it follows that this *Blastomycete* caused among the inoculated animals neoplastic growths of a malignant character.

Observations have been made by Roncali¹³ upon *Blastomycetes*, but only from a histological standpoint. He found them in the cytoplasm of certain cells, similar to those of a *corpus luteum*, which were present in the stroma of an adenocarcinoma of the ovary. The tumor, the size of a child's head, consisted of three cysts, which contained a slimy fibrous fluid. On the inner wall of the cysts, and floating in the fluid, there were found little bodies of different sizes with a cauliflower appearance. Histologically, these neoplasms consisted of a ground-work of connective tissue, from which there were numerous ramifications lined with cylindrical epithelium. In the enlarged cell bodies of the epithelium were found the parasite. The cell protoplasm was granular and the nucleus usually apparent. In some cells the nucleus was pushed to one side and hardly distinguishable, and in others the protoplasm appeared as if vacuolated. The parasites in great numbers were both within the cells and free in the connective tissue, most of them, however, in the former condition. Very many of the cells contained a single parasite,

though some of them had as many as ten. These cells were swollen, with the nucleus shoved to one side. The form of the parasites was round, rarely oval or kidney-shaped. They varied in size, some being larger and others smaller than the nucleus of a cell. They were always within the cell protoplasm, never within the nucleus. A capsular membrane surrounded them, which appeared as a hyaline circle. The younger forms possessed no membrane. No nucleus was found. The younger and growing forms were more numerous than the old and degenerate ones, as the latter, he considered those forms which did not stain, but appeared pale and refractive.

Twelve weeks after the operation for the removal of the adenoma, the patient died. At the autopsy many metastatic tumors of the diaphragm and omentum were found. The omentum was thickened and on its surface appeared several nodules of different sizes and of greyish-white color, which, upon being incised, appeared compact and of pearly whiteness. By microscopical examination, many parasites were found in these metastatic tumors. They were similar to those found in the primary lesion, with the exception that only a few older forms were seen. A number was extra-cellular, when they appeared in groups of from seven to thirteen, surrounded by leucocytes or epithelial elements.

Roncali failed completely in the production of pure cultures. From the histological examinations, he concluded that these cells were a variety of *Blastomyces*, because of their close identity to forms seen by the many other investigators. Naturally, Roncali comes to the conclusion that there is an etiological connection between the primary adeno-carcinoma and the secondary metastatic tumors of the omentum and the mesentery, from the fact that the parasites which were nested between the bundles of connective tissue and in the swollen epithelial cells of the tumor, are likewise found in the metastatic tumors, both in the epithelial cells and among the fibers. Another important fact is that only younger and

growing forms were present in the metastatic tumors, and the older and degenerate forms in the primary tumor.

Roncali,¹⁴ in trying to establish the etiological relation of yeast fungi to sarcoma, made histological examinations of five sarcomas. The first, a large round celled sarcoma of the crest and body of the ileum; the second and third, spindle celled sarcomas somewhat melanotic, from the orbit; the fourth and fifth, large spindle celled sarcomas of the superior maxilla, also somewhat melanotic. His results were as follows: in five tumors from different locations and in different tissues, he found in every case parasites. They resembled morphologically the forms which Sanfelice had described. They took the stains of the *Blastomycetes* and withstood the action of acids and alkalis. They were found both inside and outside of the cells. They multiplied by budding. Some were found in youthful stages without a membrane, with highly colored protoplasm; and others older, with a thick membrane and a somewhat chromatic heterogeneous protoplasm which had lost the faculty of staining.

Colpi,¹⁵ claims to have found a variety of yeasts in a case of chronic endocervicitis, which acted as the etiological agent. But from the fact that he could in no way produce fermentation with it, authorities believed either that it was not a yeast, or if it was, it represented a degenerate form.

Other Italian authors, Claudio, Fermi and Aruch,¹⁶ have worked on this subject and have concluded that there is a pathological yeast, whose chief characteristic is to produce neoplasms of a chronic nature, whose cellular elements had the property of wandering away from the neoplasm into the lymphatic glands. They isolated their yeast from a horse affected with an infectious kind of lymphangitis, and found by inoculations that it was markedly pathogenic.

Lydia Rabinowitsch,¹⁷ under the direction of Koch, has studied the action of fifty different varieties of yeast on test animals. Of these only seven were found to be pathogenic, and none of those were from culture yeasts. Inoculations of the culture yeasts proved fatal in mice and rabbits only after

immense doses, and even then only a few parasites were found in the tissues.

She concludes from her research that none of the pathogenic varieties were identical to those which had been described by the other authors. In her cases it is probable, from the fact that the yeasts were more plentiful in the blood than in the organs, that death was due to the infection. In the dead animals most of the cells were found free in the tissue, only a few being seen in the cell elements.

On a gelatine plate culture of pus, taken from an appendix removed by Professor Carmalt, a colony of yeast fungi was found, which the author studied. In gelatine stab culture the growth appeared in the form of the needle along the whole stab. On gelatine plates they formed a small white globular colony, which spread very slowly without liquefying the gelatine. On slant agar the colony, which spread more rapidly than on gelatine, presented a pearly white appearance. On potato the growth was quite luxuriant, giving a yellowish-white appearance. Examined in the hanging drop, the cells were found to be mostly round or oval, consisting of a granular mass of protoplasm, which was surrounded with a double contoured membrane. Stained with methylene-blue, the protoplasm of the cell showed deeply-stained granules. Because of the lack of material, no inoculations were made to ascertain whether the yeast was pathogenic. Consequently the results are of little value ; however, had a like discovery been made two years ago it would have been regarded as an accidental inoculation from the air. Now, with the knowledge that yeasts have been found in the pus of a sarcomatous sub-periosteal tumor and in a sarcoma accompanied with *Ascites chylosus*, there is reason to believe that this specimen of the yeast fungi, when inoculated into test animals, may prove to be pathogenic.

Reviewing the whole field of research on this subject, what conclusions can be drawn, first, as regards the yeast fungi in their relation to animal and human tissues ; and second, in regard to their relationship to malignant neoplasm ?

(a.) Yeast fungi are known to invade the tissues of animals and men, where they subsist in the same manner as bacteria.

(b.) Certain varieties of yeast fungi are pathogenic through infection to animals, among which are dogs, pigeons, horses, cats, rabbits, guinea-pigs and mice.

(c.) They are present in diseased conditions of the human tissue.

Before trying to establish any relationship between the yeast fungi and malignant tumors, it is necessary to review, with that idea in mind, the work of the several investigators.

The investigation of Roncali is, perhaps, of the least real value in this problem, because he dealt with the question from a histological standpoint only. His case of adeno-carcinoma of the ovary with metastatic tumors in the mesentery, presented what he believed to be yeast fungi. The fact that they are fungi he infers from their marked similarity in shape, size and appearance under the influence of stains, to the forms which Sanfelice has proven to be yeasts. His investigation of the five cases of sarcoma was of the same nature. In all cases he found bodies identical to those which other authors have isolated from sarcoma, inoculated into test animals, and caused tumors similar histologically to the original one.

Büsse succeeded in isolating a pure culture of a yeast, from a sarcomatous sub-periosteal tumor of the tibia. This he found to be pathogenic to animals, and in the case of an inoculated dog to cause a tumor. His observations were not very complete, as he published his paper before the tumor had been examined histologically. Although there is no direct evidence as to what the tumor was, yet we are warranted in inferring, from results obtained by other men, that if it had been examined the presence of yeast fungi would have been determined.

The observations of Maffucci and Sirleo are of importance, because they succeeded in producing neoplasms in all of their test animals, for which their fungi were pathogenic. They

were among the first to suggest the possibility of the yeast fungi being the cause of malignant tumors.

Corcelli and Frisco present results of the utmost importance. They succeeded in procuring a pure culture of a *Blas-tomycete* from a tumor, which, both during life and at the autopsy, showed itself to be a malignant neoplasm. This caused, among the test animals, tumors which were identical to the original tumor, both in location and structure. It is certainly easy to establish an etiological relationship here. Furthermore, with the discovery of this parasite, equipped with the power to cause malignant tumors, there is opened a new field in the study of *Ascites chylosus* and the connection which exists in man between these tumors and the exudate.

To Sanfelice we are indebted, more than to any other investigator, for our present knowledge of pathological yeast fungi. The uniformity of his success in producing tumors among the test animals with all of his pure cultures is most remarkable. This fact, together with the notable similarity of the yeasts in the tissues of the animals which had been inoculated, to the so-called *Coccidia* of many authors on the cause of cancers, led him to investigate malignant growths, with the result that he succeeded in making pure cultures of yeasts from a case of carcinoma of the breast and one of carcinoma of the lip. These cultures, by inoculation, proved pathogenic.

The results of Lydia Rabinowitsch, who found only seven pathogenic varieties of yeast among fifty, which in no case produced tumors, somewhat temper the results of the other investigators. None of her yeasts produced neoplasms, and none of them were pathogenic to guinea-pigs although to other animals. These results are not of so much importance when we consider that of all the yeasts known four years ago only two varieties were found by Sanfelice to be pathogenic.

The results of Fermi and Aruch, who produced a pure culture from a horse affected with an infectious lymphangitis and proved it pathogenic, add evidence not only to the patho-

genic character of yeast fungi but also to their ability to produce malignant growths.

Almost all the investigators of yeast fungi have alike been impressed with the marked similarity which exists between the yeasts as they appear in tissues and the numerous forms of cancer bodies, as they have been described and pictured by the many writers on the parasitic nature of malignant tumors, especially carcinoma. With this fact in view, it seems best to consider them here and, if possible, to show that they represent in the tissues a variety of yeasts.

After the suggestion offered by Paget, in 1887, at various times, several authors, as Rappin,¹⁸ Scheurlein,¹⁹ Brault,²⁰ Seneger,²¹ Baumgarten,²² Rosenthal²³ and Kubasoff²⁴ described varieties of bacteria as the specific cause of cancer, but the discovery of similar microorganisms in non-cancerous tissue led to the belief that their presence in those cases was merely an incident.

The apparent protozoan nature of certain bodies in epithelial formations, such as *Molluscum contagiosum*, described originally by Virchow²⁵ and a number of other investigators, among them Rivolta,²⁶ Bollinger,²⁷ Neisser,²⁸ suggested the likelihood of the existence of cancerous bodies in the allied cancerous formations.

In 1888, Pfeiffer²⁹ described Sporozoa, and Malassez,³⁰ in 1889, announced the discovery of bodies resembling *Coccidia oviformes* in the cells of the epithelioma of the jaw.

About the same time Thoma³¹ described small cell-like bodies found in the epithelial cells of glandular cancers of the rectum, breast and stomach, all of which, and especially the latter, might be, from their description, easily confounded with yeasts.

Albarren³² found round bodies in the epithelia, which he described as psorospermes, a greater part of which he says had the form of round or little egg-shaped cells, also possessing a single central nucleus. Some of the parasites were clearly encapsulated with a membrane of varying thickness. There were others which were very refractive and homogeneous with-

out nuclei. One could scarcely need a better description of the *Blastomyces* found by Sanfelice in the tumors of the guinea-pigs which had been inoculated with a pathogenic variety of yeast fungi. The latter part of the description tallies precisely with the form which the degenerated *Blastomyces* take on.

Darier³³ found, in a certain skin disease, *Psorospermiosis follicularis vegetaris*, round, intra- and extra-cellular bodies with a thick refractive membrane. His figures correspond exactly with drawings of yeasts.

Wickham, a scholar of Darier, found similar bodies in cases of Paget's disease. His drawings also were like those of yeasts.

Nils Sjöbring,³⁴ who had been studying the process of nuclear division in cancer cells, had noticed certain bodies which he called Protozoa. The cycle of development, so well described and pictured by him, can be easily explained by stages in the development of the yeast fungi.

These descriptions of Albarran and Sjöbring were soon corroborated in publications of Balbiani,³⁵ Hacks,³⁶ Wright,³⁷ Strobe,³⁸ Steinhaus³⁹ and Kursteiner.⁴⁰

Soudakewitch⁴¹ found in the cells and cell nuclei of glandular cancers small round or oval bodies, sometimes with a membrane about them. Metchnikoff, who also examined them called them *Coccidia*. His drawings are very similar, in some respects, to those of authors on *Blastomyces*.

Sawtschenko and Podwyssozki⁴² failed to notice parasites within the cell nucleus, but in most cases found them in the cell bodies.

Sawtschenko⁴³ described the apparent life history of the so-called Sporozoa from sections of a cancer of the lip. Here, also, both the descriptions and drawings of these stages resembled very closely conditions of the yeasts.

Foa⁴⁴ studied especially carcinoma of the breast and mentioned small bodies, which were usually homogeneously but sometimes contained deeply-stained central granules, also larger forms, which might reach the size of a white blood

corpuscle. They were surrounded with an intensely stained capsule. He also described forms which, had he not been looking for *Coccidia*, might have been explained as *Blastomyces* in the process of budding. Other forms, which present a striking resemblance to these, were those which he described as not staining. Whether this was due to degeneration he was unwilling to say.

Koretneff,⁴⁵ Kurloff,⁴⁶ Smith⁴⁷ and Clarke⁴⁸ describe forms of a gregarine-like body, which they claim is the fully developed form of the parasite, and to which the name of *Rhopalcephalus carcinomatosum* was given. An undeveloped stage of this parasite, which was found most frequently in the sections, resembles very much the yeast fungi.

These are the only writers who have recognised gregarine-like forms as a factor in the etiology of the epithelioma. From the fact that they were found only in relation to pearls of epitheliomata of the lip or penis, it seems as if they had mistaken degeneration products for genuine organisms.

Buchardt⁴⁹ studied cancers of the mucous membrane and found in most of them intracellular structures surrounded with a clear capsule.

Ruffer and Walker⁵⁰ confirmed the investigation of continental workers and, later, Ruffer⁵¹ announced that he had seen every stage in the life history of the Protozoon of cancer, from the time the parasite appeared as a spore in the nucleus until it left the latter as a young, fully formed parasite.

Galloway⁵² has recently announced his belief in the protozoan nature of the various bodies described, on account of their apparently organised structure, their staining reactions and their analogy to well-known Protozoa. He figures them as small round masses of protoplasm, which may become encysted and then break up into a number of sporules.

Of all the authors who have busied themselves with the parasites of malignant tumors, Russel⁵³ is the only one who has described a fungus as the real cause. These, because of their affinity for acid-fuchsin, he calls "fuchsin-bodies," and states that they may be found in cancers without exception.

These bodies may occur in groups of two, three, four or more ; generally have a clear space about them ; are bounded by a capsule ; are spherical in shape, measuring from four to twelve mikrons in diameter, and are structureless in appearance. He judged that they grew by gemmation. They were found in the more rapidly growing part of the tumor.

Realising the weakness of a parasitic theory of the origin of cancers, which is unable to comply with Koch's laws, several investigators have attempted to inoculate cancerous tissues into test animals. Hanan made a successful inoculation of carcinoma taken from the vulva of one rat into that of another, producing not only a similar tumor but also metastasis. Fuschel tried inoculation experiments, but failed. Klebs, in his inoculation experiments, found that the parts were absorbed. Power⁵⁴ assumed that the parasites, as in malaria, were propagated in soil, and collecting soils from districts which were evidently cancer districts impregnated them with cancerous tissue. On these, white rabbits, which had been subjected to irritation on different parts of their bodies were compelled to live for some time. His results were also negative.

Pathologists have succeeded in finding parasites in sarcoma as well as in carcinoma.

Vedeler,⁵⁵ after the study of four sarcomas, comes to the conclusion that probably the numerous cell enclosures which he found were Sporozoa. His drawing of the stages of their development compared with those found by Büsse are very significant ; and, with the description of yeasts by Corselli and Frisco, cause one to be impressed with the marked similarity of forms which were found by those authors, both in the original tumor and in the tumors produced in the test animals.

Pawlowsky,⁵⁶ in his study of fourteen cases of sarcoma, came to the conclusion that the parasites which he found had an etiological connection with the spreading and growth of the tumors. In the description of the life history of the parasites, he maintained that the spore was the starting

point. His drawings also show strong resemblance to the yeast fungi described by Corselli and Frisco, as well as a similarity in form to those shown by Büsse.

Steinhaus, Hadden, Wernicke, Jackson and Clarke are other pathologists who have recognised similar parasites in sarcomas.

In the author's investigation of tumors the specimens were prepared as follows : Taken in most cases absolutely fresh, and in some still life-warm, they were cut into pieces, five cubic centimeters in size, and placed in a saturated solution of corrosive sublimate at least three hours, but no longer than twenty-four. (The fixing solution was prepared by adding to boiling distilled water as much corrosive sublimate as would dissolve. Upon cooling the supernatant fluid was not decanted, but placed in a dark bottle. The solution will keep only two weeks, and must be placed where it is cool.) After being washed they were removed to 70 per cent. alcohol, where, with daily changes, they remained a week. The strength was then increased daily to 80, 95 and absolute alcohol. Then the specimens were cleared in oil of Bergamot for twenty-four hours, then in xylol twenty-four hours, and finally placed in xylol and paraffine, and paraffine alone. The sections were cut as thin as possible. Besides the stains of eosin and hematoxylin, the Ehrlich-Biondi triple stain, safranin and malachite-green, lithium-carmin and Ehrlich's fluid with gentian-violet were used ; but the most beautiful results were derived from the following method : Place the slide for twenty-four hours in a dilute solution of Delafield's hematoxylin (2 per cent. is found strong enough), wash in water twenty-four hours, changing frequently ; leave in gentian-violet and aniline-water twenty-four hours, wash thoroughly ; differentiate quickly in acid alcohol, then a moment or two in eosin alcohol ; dehydrate quickly in absolute alcohol, clear in xylol and mount in balsam.

With a few exceptions, parasites were found in all the specimens examined. These included specimens from eighteen schirrus cancers of the breast, five recurrent cancers

of the same variety from the same region, two cancers of the penis, one from the upper lip, one schirrus cancer from the mesentery and one from the stomach, one round-celled sarcoma of the stomach and one of the breast. Of these, the results of the research in a few selected tumors will be taken to represent the study of the whole.

In a case of carcinoma of the penis there were seen numerous cell enclosures, which stained similarly to yeast fungi found in cancerous tissue by Sanfelice and Roncali. Some were found in vacuoles and others showed no differentiation from the cell contents but that marked by the stain. A few were found which presented deeply-stained granules. Many of them were so small that they did not change the contour of the nucleus, while a few were of such proportions that the nucleus was not only crowded to the periphery of the cell but was pressed into a crescent shape. Besides those bodies which appeared as real parasites there were found numerous other bodies of forms similar to those described by some of the authors mentioned above. Among these forms were several specimens similar to the so-called *Rhodalocephalus Carcinomatosum*, of Koretneff, which bodies, from the appearance presented in the sections of the writer, could be more readily explained as products of degeneration than as parasites. Steinhaus has described the process by which the cells are gradually pressed together until they become degenerated masses, which upon section may present many different shapes that might be taken for parasites. In this case of carcinoma of the penis the arrangement of the cells about these masses suggested the existence of pressure, the cells in contact with the mass were very thin, much like the superficial cells of the epidermis, there remaining only the evident outline of the cell, without a nucleus. The next layers of cells were thicker, and many of these possessed distorted nuclei, and finally, toward the periphery, perfectly normal cells were seen. Bodies were found which corresponded very closely to some of those described and drawn by Foà, which seemed more like cell invaginations.

Many parasites were found in the schirrus carcinoma of the breast. These were similar in form and size to those drawn by the various authors who have described the yeasts.

In a case of schirrus carcinoma of the stomach and the metastatic tumors from the axilla, parasites were found, which were identical in form, size and in their reaction to staining reagents. They were uniformly round, homogeneously stained and situated within vacuoles. In a case of a round-celled sarcoma of the stomach, besides forms similar to those mentioned above, there was found a form which was undergoing what seemed to be a process of budding, being similar to those forms described by Roncali in his research.

In comparing the yeast fungi and the parasitic bodies of malignant tumors, something ought to be said in regard to the staining reactions of the latter. Opponents of the parasitic theory claim that all of the appearances presented by the so-called Protozoa can be explained by the staining reactions of the different kinds of cell metamorphoses, as hyaline, colloid, gelatinous and horny degenerations; or by irregularities of cell life, as cell invagination, vacuolisation, dropsy of cell bodies, irregularities in the distribution of chromatin and dispersion of chromosomes, fragments of such during and after the karyokinetic process and asymmetrical mitosis, or they can be explained by the inter- and intracellular invasion of leucocytes and red blood corpuscles. While their statements may be true in the case of many forms which has been presented as parasites—for, at one time or another, no doubt, every one of the irregular forms mentioned above have been pictured among parasites—yet a greater part of them cannot be explained away by any method of staining. In fact, our knowledge of the reactions of the different products of degeneration toward stains is so meager that neither side can disprove claims of the other in many cases. A specific stain has not been found for Protozoa or yeast fungi, so that staining reactions are of little value in procuring evidence in support in either class of bodies.

Most of the forms, as we have seen, which have been

described by investigators as cancer bodies, can as readily be considered as yeast fungi. As regards the remaining forms, it may be said that, in order to obtain the knowledge that they were dealing with Protozoa, they were compelled to hunt for forms which were not present, and consequently christened as such bodies which were not parasitic.

Finally, in drawing our conclusions as to the relationship of these fungi to the malignant tumors of man, we may say that :

First—From a histological standpoint, we probably have as evidence the results of the investigations of those who, for the past eight years, have upheld the parasitic theory of malignant tumors, men who have seen yeast fungi, but have incorrectly called them Protozoa.

Second—From the researches of Sanfelice, Corselli and Frisco, and Büsse, that yeast fungi have been found in man and the lower animals affected with malignant tumors. These, isolated, grown on nutrient media and inoculated into other animals gave rise to malignant tumors, which showed the same characteristics as the original tumors.

The field of research is but just opened and the investigations confined to a few centers of learning ; still, with the main obstacle overthrown—namely, compliance with the laws of Koch, it seems that we are much nearer to the solution of that difficult problem, the etiology of malignant growths.

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